



Zika Virus Sample Collection Guidance

General information:

This document provides sample collection instructions for Zika virus testing. For any questions regarding specimen collection or shipment for Zika testing, please contact the DC Public Health Laboratory (PHL) at Zikalab@dc.gov.

Serum specimen:

For adults:

- Collect two tubes of serum (containing NO anticoagulant or preservative) with at least 6 milliliters (mL) of blood per tube (e.g., red top, tiger top, speckle top, gold top, or other serum separator tube). Do NOT use any blood collection tubes containing anticoagulants or preservatives (e.g., green top, yellow top, or purple top).
- After collection of whole blood allow the blood to clot by leaving it undisturbed in the laminar flow hood for 15 – 30 minutes, but not longer than 1 hour. **NOTE:** Please be sure to follow blood collection tube manufacturer's instructions on how to properly allow for clot formation, if different than these instructions.
- Promptly (within 1 - 6 hours) centrifuge serum according to manufacturer's instructions. Use of serum separator tubes is recommended.
- A minimum of 3mL of serum is recommended for each tube of blood drawn.
- Using sterile laboratory technique, transfer the serum into a sterile tube using a sterile transfer pipette. The sterile tube should then be sealed in Parafilm M to prevent spillage. The transfer pipette should be discarded in a biohazard bag.
- **NOTE:** The original vacutainer tube may also be sent and must also be appropriately sealed with Parafilm M as well.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, and date and time of collection, and specimen type (serum).
- Serum samples are preferred to be stored in an ultra-low temperature freezer and maintained at -70°C while awaiting sample pick-up by the DC-PHL courier. If a -70°C freezer is unavailable, then samples may be stored in a -20°C freezer or at a minimum 4°C refrigerator but must be delivered to the DC PHL within 72 hours.
- Specimens that leak will not be tested.



For infants:

- Collect a minimum of 1mL of serum per tube (red top, tiger top, speckle top, gold top or other serum separator tube) using a venous sample, NOT a heel stick. Do not use any blood collection tubes containing anticoagulants or preservatives.
- After collection of whole blood allow the blood to clot by leaving it undisturbed in the laminar flow hood for 15 minutes, but not longer than 45 minutes. **NOTE:** Please be sure to follow blood collection tube manufacturer's instructions on how to properly allow for clot formation, if different than these instructions.
- Promptly (within 1 hour) centrifuge serum according to manufacturer's instructions. Use of serum separator tubes is recommended.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, and date and time of collection, and specimen type (serum).
- Serum samples are preferred to be stored in an ultra-low temperature freezer and maintained at -70°C while awaiting sample pick-up by the DC-PHL courier. If a -70°C freezer is unavailable then samples may be stored in a -20°C freezer or at a minimum 4°C refrigerator but must be delivered to the DC PHL within 72 hours.
- Specimens that leak will not be tested.

Urine specimen:

For adults:

- A urine **and** serum sample needs to be collected during the same patient visit, packaged together, and sent for testing. Urine samples alone **will not** be tested.
- Collect 5-20 mL of urine in a sterile screw-top container. Please do not over fill the sterile container with urine as only 5-20 mL of urine is required for testing. The sterile container should then be sealed in Parafilm M to prevent spillage.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, date and time of collection, and specimen type (urine).
- Place the sealed urine container in a specimen transport bag; seal the bag. Then place the sealed transport bag into a second specimen transport bag with the same patient's serum specimens. Seal the second (outer) bag containing both urine and serum specimens. Place the test requisition form in the sleeve of the outer bag.
- Urine samples are preferred to be stored in an ultra-low temperature freezer and maintained at -70°C while awaiting sample pick-up by the DC-PHL courier. If a -70°C freezer is unavailable then samples may be stored in a -20°C freezer or at a minimum 4°C refrigerator but must be delivered to the DC PHL within 72 hours.
- Specimens that leak will not be tested.



For infants:

- A urine **and** serum sample needs to be collected during the same patient visit, packaged together, and sent for testing. Urine samples alone **will not** be tested.
- A minimum of 1.0 mL of urine should be collected in a sterile screw capped container and sealed with Parafilm M to prevent leaking.
- Label the container with the patient's name (last, first), date of birth, Zika ID, date and time of collection, and specimen type (urine).
- Urine samples are preferred to be stored in an ultra-low temperature freezer and maintained at -70°C while awaiting sample pick-up by the DC-PHL courier. If a -70°C freezer is unavailable then samples may be stored in a -20°C freezer or at a minimum 4°C refrigerator but must be delivered to the DC PHL within 72 hours.
- Specimens that leak will not be tested.

Cerebrospinal fluid (CSF) specimen:

- All CSF samples must be accompanied by a serum sample to be eligible for testing.
- Collect CSF within 7 days of symptom onset.
- It is recommended that 3 mL of CSF be collected, however at least 1 mL CSF is required for testing. Only the second through fourth tubes of CSF should be sent due to the potential of blood in the initial tube.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, date and time of collection, and specimen type.
- Seal the sterile tube with paraffin wax and place in specimen biohazard bag with absorbent material.
- For antibody and rRT-PCR testing, specimens should be kept cold (2–8°C).
- Specimens with blood may be rejected.

Other body fluid specimen:

Amniotic fluid:

- Collect 3-5 mL of amniotic fluid in a sterile screw-top container (15 mL centrifuge tube). Use paraffin wax to seal.
- Label the tube with the patient's name (last, first), date of birth, Zika ID, date and time of collection, and specimen type.
- Place the tube in specimen collection bag with absorbent material.
- Amniotic fluid specimens should be kept frozen (-70°C) for storage and shipping.



Tissue Specimens (placental tissue, umbilical cord tissue)

- For placenta and fetal membranes, several full thickness pieces including **at least 3** full thickness (0.5-1cm x 3-4cm in depth) from middle third of placenta disk AND **at least 1** from the placenta disk margin and one 5x12 cm strip of fetal membrane should be sent.
- All tissues need to be fixed in formalin. The volume of formalin used to fix tissues should be 10x the volume of tissue. Place tissue in 10% buffered formalin for a minimum of three days or until fully fixed. After fixation, tissue can be transferred to 70% ethanol for long term storage.
- If stored prior to shipping, please transfer fixed tissues to 70% ethanol after 72 hours.
- Fixed tissues should be stored and shipped at **room temperature** (ambient).
- Paraffin blocks should be submitted in accordance with these instructions for formalin-fixed specimens.
- **DO NOT FREEZE samples that have been fixed in formalin.**
- For further instructions on sending tissue samples, please refer to <http://www.cdc.gov/zika/laboratories/test-specimens-tissues.html>.

Specimen labelling:

Please be sure to properly label **ALL** specimens. Failure to properly label a specimen will result in rejection and the specimen will not be tested. Specimens **must** be labeled with:

- Patient's first and last name
- Patient's date of birth
- Zika ID
- Date and time of collection
- Specimen type (e.g., serum, urine, CSF, amniotic fluid)

All information on the specimen labels must **exactly** match the information on the PHL test request form, including the spelling of the patient's first and last names and date and time of collection. Specimens received with discrepant information on the labels and requisition forms will not be tested.

*Specimen storage and shipping:

- Specimens for Zika testing (except formalin fixed tissues and CSF) are optimally stored at -70°C (ultra-low temperature) after specimen collection to maintain integrity of the sample. Freezing specimens at -20°C temperatures is acceptable. Minimally, refrigeration at 4°C is allowable (but not preferred) for up to 72 hours. With the exception of formalin fixed tissues, specimens should **never** be left at room temperature for extended periods of time, especially overnight.



- All specimens (except formalin fixed tissues and CSF) should be stored at -70°C if not picked up within 72 hours (e.g., sample collection was completed on a Friday afternoon and pick-up will not occur until Monday).
- Serum and urine specimens not stored at -70°C need to arrive at the DC PHL within 72 hours for testing to be accepted. If these specimens arrive after 72 hours and were not frozen at -70°C , then it may be rejected.
- If a -70°C freezer is not available at your facility, please store specimens the freezer at -20°C and indicate this on the courier pick-up email.
- If a freezer is not available at your facility (either -70°C or -20°C), please store specimens in the refrigerator at 4°C and indicate this on the courier pick-up email.
- If a refrigerator or freezer is not available at your facility, please indicate this on the courier pick-up email and indicate that this is an **URGENT** request for Zika specimen pick-up. A courier will need to be dispatched to you immediately to pick up the specimens.
- **NOTE:** The DC-PHL is only open from Monday through Friday from 9am to 5pm, and closed for all federal holidays. Therefore if a refrigerator or freezer is not available at your facility and a patient is scheduled to come in on a Friday or before a holiday it is recommended to schedule patient collection dates appropriately to ensure the specimen is received by the DC-PHL. **In these cases, samples should be collected as early as possible Friday morning and the request for sample pickup should not occur past noon on Friday, especially before a holiday weekend.** This will allow sufficient time for the courier to pick up the specimens and deliver them to the public health laboratory.
- **NOTE:** It is important that frozen samples not thaw during shipping. To prevent this, be sure to ship with dry ice and follow proper Department of Transportation (DOT) and International Air Transport Association (IATA) shipping regulations. If no dry ice is available at your facility, please indicate this on the courier pick-up request email that is sent to Zikalab@dc.gov.
- **NOTE:** For unique (not serum/urine) specimens (e.g., CSF, amniotic fluid, placental tissue, etc.), please call the DC Department of Health (DOH) to discuss proper handling of specimens.
- Specimens that are deemed to be improperly stored, packaged, shipped, and/or labelled may be rejected at the discretion of the DC Public Health Laboratory.

Additional Testing:

- Positive (reactive) IgM results by ELISA will be confirmed by testing for neutralizing antibodies. Plaque-reduction neutralization tests (PRNTs) can be performed to measure virus-specific neutralizing antibodies and may be able to discriminate between cross-reacting antibodies in primary flavivirus infections.
- The DC PHL does not currently perform PRNT or IgM dengue serology testing and will forward all approved specimens to the Centers for Disease Control and Prevention (CDC) for follow-up IgM dengue and confirmatory PRNT testing.



REQUIRED DOCUMENTATION FOR TESTING AT THE DC PHL

- DC Public Health Laboratory Test requisition form
- DC Public Health Laboratory Chain of Custody form

Note: If the proper paperwork is not complete upon pick-up, the courier has the right to refuse the sample. Please do not email for pick-up until all forms are complete.

For questions regarding sample collection, storage, packaging, and shipping, please contact the District of Columbia Public Health Laboratory at (202) 727-6929